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Evolutionary tales of maskrays (*Neotrygon*, Dasyatidae), flatheads (Platycephalidae, Scorpaeniformes) and tuskfishes (*Choerodon*, Labridae).

by
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DECLARATION OF ORIGINALITY

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STATEMENT OF ETHICAL CONDUCT

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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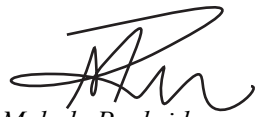

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PUBLICATIONS

Chapter 2

Puckridge M, Last PR, White WT, Andreakis N (2013) Phylogeography of the Indo-West Pacific maskrays (Dasyatidae, *Neotrygon*): a complex example of chondrichthyan radiation in the Cenozoic. *Ecology and Evolution* **3**, 217-232.

Chapter 3

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Supplementary publications

Last P, White WT, Puckridge M (2010) *Neotrygon ningalooensis* n. sp. (Myliobatoidei: Dasyatidae), a new maskray from Australia. *Aqua, International Journal of Ichthyology* **16**, 37-50.

White WT, Last PR, Dharmadi, Faizah R, Chodrijah U, Prisantoso BI, Pogonoski JJ, Puckridge M and Blaber SJM (2013) *Market fishes of Indonesia (=Jenis-jenis ikan yang di Indonesia)*. ACIAR Monograph No. 155. Australian Centre for International Agricultural Research: Canberra.

STATEMENT OF CO-AUTHORSHIP

The concept development, survey design, implementation, production, analysis and interpretation of data, writing and final editing of the manuscripts were the responsibility of the primary author and PhD candidate, M. Puckridge. Additional input from co-authors was as follows:

For Chapter 2:

P.R. Last helped develop ideas, chose the focus group and provided editorial input to refine the manuscript.

W.T. White provided the great majority of tissue samples as well as editorial input to refine the manuscript.

N. Andreakis helped develop ideas, provided guidance with analyses, their interpretation and manuscript layout as well as editorial revisions.

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N. Andreakis provided guidance with the analyses and editorial input to the manuscript.

S.A. Appleyard offered general laboratory advice as well as editorial input to the manuscript.

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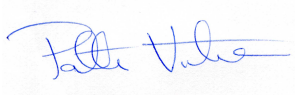

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ABSTRACT

The tropical Indo-West Pacific (IWP) is the most biologically diverse marine region on earth with a number of competing hypotheses proposed to explain the evolutionary events responsible for this area's biotic complexity. These hypotheses are based on varying interpretations of species distributions radiating from the Indo-Australian Archipelago (IAA) at the centre of the region. The IAA represents a geologically dynamic area formed by colliding tectonics; mechanisms relating to tectonic activity are therefore commonly considered responsible for the biodiversity here.

In the present study, a) maskrays of the genus *Neotrygon* (Dasyatidae), b) flatheads of the family Platycephalidae (Scorpaeniformes) and c) tuskfishes of the genus *Choerodon* (Labridae) were studied with respect to their taxonomy, phylogeny and phylogeography. These groups are characterized by having demersal life histories and a high proportion of narrow-ranging endemics. Furthermore, some of the widely distributed species across the region are suggestive of either recent jump-dispersal events or fragmentation of once pan-tropical populations. The sampling effort in this study focused on exploring diversity among species, and within selected taxa, by obtaining multiple individuals across species' distribution ranges, where possible. Molecular phylogenies, coupled with molecular clock approximations, were employed to a) assess nominal species validity b) DNA barcode Evolutionarily Significant Units (ESUs) and c) explore the geological and evolutionary processes responsible for observed trends in diversity and species distribution in maskrays, flatheads and tuskfishes. Both mitochondrial and nuclear molecular markers were used in this study. The chosen markers amplified consistently across species, showed high content of phylogenetic signal and were able to resolve closely related species, thus allowing the inference of robust phylogenies and molecular clock dating.

Parallel genealogical trajectories have been recovered within rays of the genus *Neotrygon* and platycephalin flatheads (the most comprehensively sampled subfamily of Platycephalidae). These congruent spatial and temporal patterns suggest that species differentiation predominantly occurred across the tropical IAA throughout the mid to late Miocene. The most widely distributed and derived forms in these groups – *Neotyrgon kuhlii* and *Platycephalus indicus* – were found to consist of cryptic species complexes with considerable lineage diversity across their IWP range. Phylogeographic comparisons with other, less well sampled platycephalid species confirm that diversification patterns are consistent across multiple species, suggesting species diversity in shallow water marine fauna may be grossly underestimated. In contrast, tuskfishes of the genus *Choerodon* showed unclear phylogeographic structuring. However, mixed ancestral and derived lineages, endemic to Australian waters, suggest the Australian region has acted as both a refuge for lineage survival and source of radiation in this group since the mid-Miocene. Patterns are

consistent with centrifugal speciation, a process that may be common across marine groups here.

In conclusion, the tectonic suture zones across the Australian and Eurasian Plates have played an essential role in triggering the area's mega-diversity and biotic complexity. Tectonic rafting, the establishment of new habitats in the form of shallow seas, tropical coastlines and island arcs, together with glacio-eustatic sea level oscillations, have likely favoured rapid species diversification through vicariance and allopatric speciation. The imprint of these large-scale geological and climatic events has been retained within the evolutionary history of maskrays, flatheads and tuskfishes. These uncovered patterns may represent a common trend for many other marine taxa with similar ranges and geographical distributions.

ABBREVIATIONS

16S	Ribosomal RNA (mitochondrial marker)
ANFC	Australian National Fish Collection
Aus	Australia
BBM	Bayesian Binary MCMC analysis
BI	Bayesian inference
BOLD	Barcode of Life Database
COI	Cytochrome oxidase subunit 1 (mitochondrial marker)
DNA	Deoxyribonucleic acid
EAC	East Australian Current
ENC1	A single-copy ectodermal-neural cortex I-like protein (nuclear marker)
GTR	General time reversible model of evolution
HKY	Hasegawa, Kishino and Yano model of evolution
hLRTs	Hierarchical likelihood ratio tests
HPD	High posterior density
IAA	Indo-Australian archipelago
ILD	Incongruence length difference test
Indo	Indonesia
IWP	Indo-West Pacific
K2P	Kimura-2 Parameter model of evolution
Ma	Million years ago
MCMC	Markov chain Monte Carlo
ML	Maximum likelihood
MP	Maximum parsimony
MRCA	Most recent common ancestor
mtDNA	Mitochondrial DNA
My	Million years
nDNA	Nuclear DNA
NGS	Next Generation Sequencing
NSW	New South Wales, Australia
PCR	Polymerase Chain Reaction
Qld	Queensland, Australia
RAG 1	Recombination-activating gene 1 (nuclear marker)
RAG 2	Recombination-activating gene 2 (nuclear marker)
TBR	Tree bisection reconnection (a branch swapping algorithm)
TIMef	Transition model of evolution with equal base frequencies
Tmo-4c4	Titin-like protein (nuclear marker)
TrN	Tamura Nei model of evolution
TrNef	TrN model of evolution with equal base frequencies
TVM	Transversion model of evolution
WA	Western Australia, Australia

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